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HISTOLOGICAL STUDY OF SPONTANEOUS EAR INFECTIONS
IN ALBINO RATS*

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SUMMARY PAGE

THE PROBLEM

Spontaneous ear infections in albino rats are not rare. The importance of histopathological investigation of rats' ears has been strongly emphasized, especially for vestibular experiments. An attempt was made to estimate the percentage of the spontaneous ear infection existing in sixteen random bred albino rats from three different supply sources.

FINDINGS

An average of 25 per cent of spontaneous middle ear infection was detected, and the common sites of the infection were found to be either in the oval window area or the round window niche.

The essential elements for chronic rat experimentation are pointed out for obtaining valid results and eliminating erroneous conclusions.

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INTRODUCTION

Spontaneous ear infection in experimental albino rats has been previously documented by several investigators (5, 7-9). In a study by Pitts (10), thirty-two albino rats were exposed to zero-G environment by the means of parabolic flight and four of the rats spun continuously. After the flight, the ears of the four spinning rats and two nonspinning ones were grossly examined. Spontaneous bulla and middle ear infections were found in the ears of those rats which spun during the flight. Because of Pitts' findings, the importance of the histopathological investigation of rats' ears has been strongly emphasized, especially for vestibular and auditory experiments.

In this experiment, therefore, an attempt was made to estimate the percentage of the spontaneous ear infection existing in random bred albino rats from different supply sources. The investigation was also extended to find the initial site or locus minoris resistentiae of the spontaneous middle ear infection in these animals.

PROCEDURE

Sixteen albino rats were used in the present study. Group I consisted of five recently purchased, three-month-old rats. Group II was composed of four rats purchased several months prior to this experiment and were approximately twelve months of age. Group III contained seven mature adult rats of unknown age. All of the rats were purchased from three separate commercial sources and were thus grouped not only according to age but also to origin.

Twisting (the tendency to turn or spin in one direction) was checked in all rats by suspending them by the tail, immediately prior to their being sacrificed under deep barbiturate anesthesia.

The temporal bones were fixed in 10% formalin by immersion, decalcified in 5% trichloroacetic acid, neutralized, dehydrated, and embedded in celloidin. The specimens were sectioned in the horizontal plane. One out of each ten sections was stained in Hematoxylin-Eosin and examined by routine microscopy (4).

RESULTS

The common clinical test for twisting revealed no spinning in all six rats subsequently found to have middle ear infections (eight ears). Two rats had bilateral infections (one side severe, the other side slight in one rat; slight bilateral involvement in the other rat). Four rats had slight unilateral infections.

Among the total of thirty-two ears investigated, eight ears (25%) were found to be infected to some extent: 1 ear, severe; 5 ears, slight; and 2 ears, very slight. The other twenty-four ears (75%) were found to be infection free.

Among ten ears which belonged to Group I, four ears (40%) were infected; three ears were slightly infected (Figures 1 and 2), and one ear was very slightly infected. The foci of the infection were around the ossicles and/or in the round window niche. The inner ear was morphologically intact except for some erythrocytes in scala tympani of the basal turn, which came through the cochlear aqueduct at the time of euthanasia.

Eight ears of Group II were void of debris or precipitate, and no infection was observed histopathologically in either the middle or inner ears.

Among fourteen ears of Group III, four ears (29%) were found to be infected: one ear was severely infected, two ears were slightly infected, and one ear was only very slightly infected. The thickening of the tympanic mucosa, adhered mucosal folds, inflammatory cell infiltration, and purulent exudate retention were noticed in the severely infected ear. The inner ear was well protected both by the annular ligament (Figure 3) of the oval window and the round window membrane (Figure 4). The inner ear was almost intact morphologically except for slight precipitate in scala vestibuli and tympani. The external ear was free of debris, and no definite perforation of the tympanic membrane was observed.

Histologically, pneumatization of the tympanic cavity is markedly well developed toward the anterior and the inferior aspect of the cavity. On the other hand, the posterior section is occupied by large flocculus cerebelli and is devoid of well-developed pneumatized structure. The anterior malleolar process is slender and long. An exceptionally large stapedial artery, which crosses between two stapedial crura, is an important anatomical distinction as it makes any surgical manipulation around this area virtually impossible (Figure 5). The possible migration of the vestibular nuclei cells (Cajal's interstitial nuclei) into the vestibular glial zone (1) is observed (Figure 6). The semicircular canals are almost embedded in a thick and compact bony otic capsule. The size of the semicircular canal crista is relatively small in comparison with that of other common laboratory animal species (Figure 7). Above all, the most important anatomical feature of this animal is the opened Eustachian tube. The orifices, both to the nasopharynx and to the tympanic cavity, are widely opened with no definite isthmus (Figure 8).

DISCUSSION

Twisting, when the rat is suspended by the tail, was absent in all six rats with middle ear infections of different degrees and combinations. In Nelson's study (7), the tympanic and petrous bone lesions were noted only with rats which showed twisting; whereas, in the present study, no distinct bony lesion was observed microscopically in such rats. The inner ear end organs might not be involved at all, or at least not to a degree which causes disequilibrium. Probably this testing is neither constant nor sensitive and is inadequate for the gradual involvement of the inner ear end organs. Immediately after acute unilateral inner ear ablation (histologically confirmed later) by the

topical injection of streptomycin sulfate, this testing showed positive confirmation (3). Inconsistency of results increased, however, when the test was repeated following the acute ablation. Therefore, this testing may be useful only for detecting the acute and severe unilateral inner ear destruction.

Of the infected ears (25% of all ears examined), the initial site of the middle ear infection in rats seemed likely to be either in the oval or the round window niche which is actually the deepest and narrowest area of the middle ear cavity. In Group I rat ears, the foci of the acute inflammation were seen only around these two niches and the surrounding stapes area.

The patency of the Eustachian tube is very important to the understanding of the relationship between nasopharyngeal infection and middle ear infection in the rat. The tube acts as a pathway which allows micro-organisms to go from the nasopharynx to the middle ear, even though the wide-opened tube is most effective for the drainage of secretion from the middle ear cavity (5).

Middle ear infection, when found in these young animals, was either slight or very slight with no exception. The high percentage of the very mild initial infection (40%) in Group I rats, accompanied by the finding of no infection in Group II rats, suggests the possibility of spontaneous cure of the initial infection. The incidence of the ear infection, according to Nelson and Gowen (8), increases with age. This, however, could not be studied in the present investigation, even though the animals represented different age groups, since the rats were purchased from different supply sources.

There was no morphological evidence of inner ear end organ involvement in eight infected ears; however, other type of inner ear involvement, such as biochemical alteration, could not be completely ruled out. The pathological findings demonstrate that both the round window membrane and the annular ligament are acting as a protection against the middle ear infection.

The rupture of the tympanic membrane was not observed in the infected ears of the present series. No clinico-otological investigation, even including x-ray examination (6), is useful for detecting middle and inner ear disease in the vital status. Thus, it is always necessary to use a considerable number of animals in each experimental group to achieve valid results and to eliminate erroneous conclusions.

Therefore, the essential elements for chronic rat experimentation are: 1) obtain rats from a good reliable supplier, 2) follow a strictly adhered program of animal care, 3) immediately isolate infected animals, 4) use carefully designed, significantly functional tests, 5) have a sufficient number of animals in each experimental group to allow for the possible existence of infected animals, and 6) confirm results histologically.

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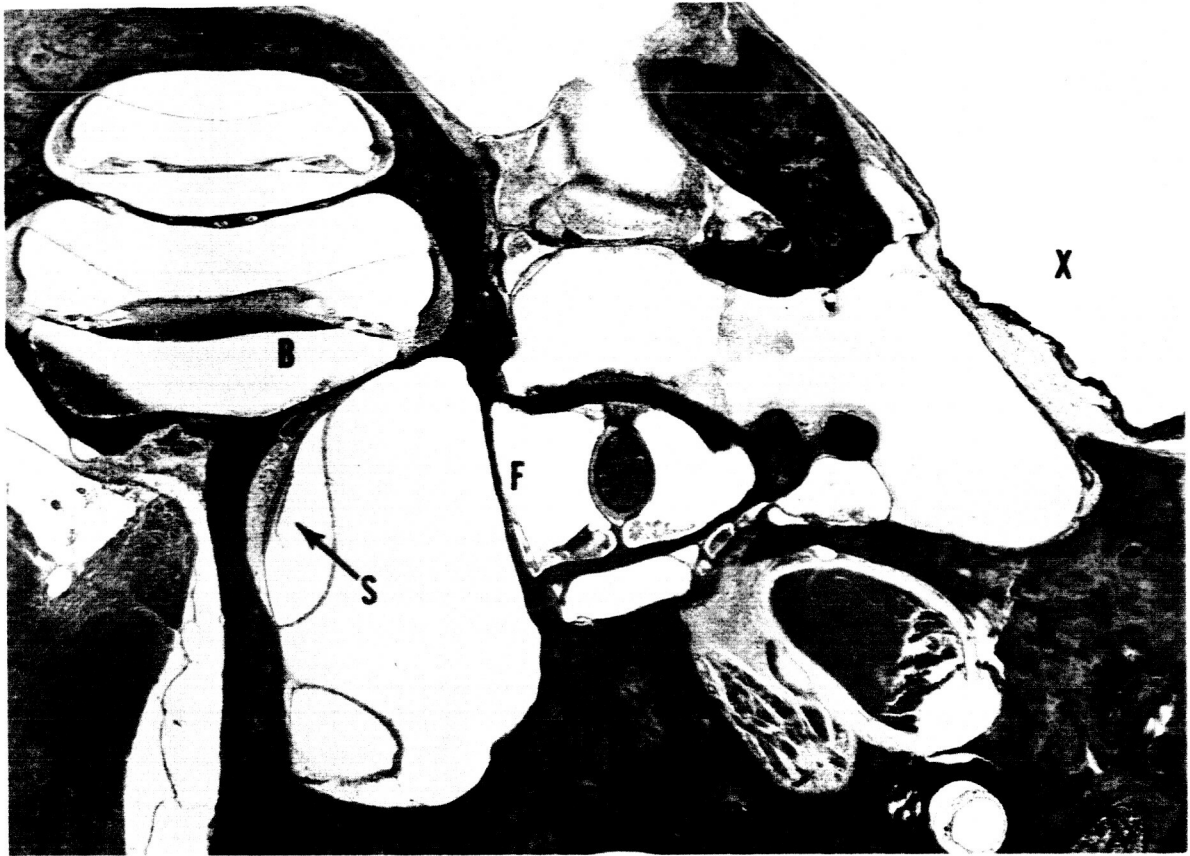


Figure 1

Microphotograph showing a slight middle ear infection in an albino rat. Note the pathology is limited around the ossicles and the inner ear is intact. Horizontal section. Hematoxylin-eosin staining. 38 X.

- X - External auditory meatus
- F - Stapes footplate
- B - Basal turn of cochlea
- S - Sacculle

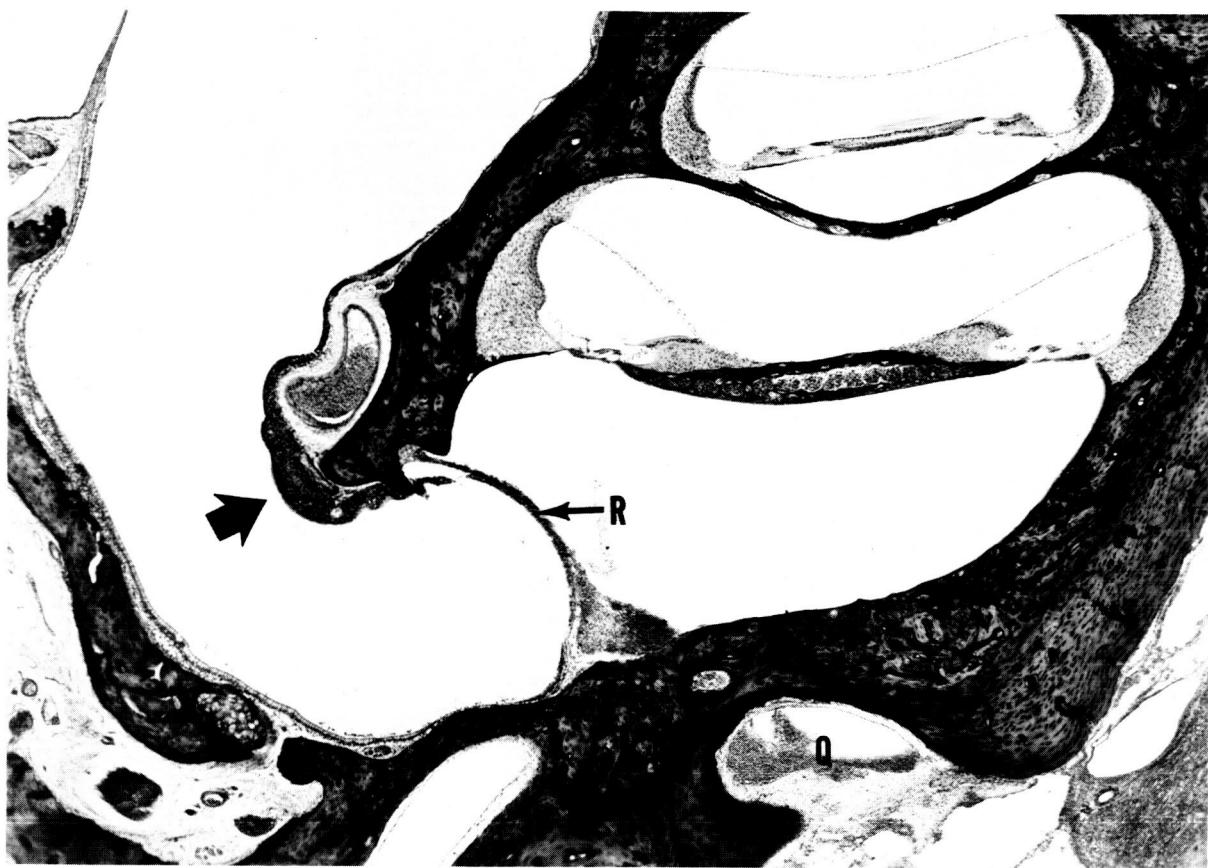


Figure 2

Microphotograph showing an infected focus in the round window niche (arrow). The inner ear is intact. Horizontal section. Hematoxylin-eosin staining. 45 X.

R - Round window membrane

Q - Cochlear aqueduct



Figure 3

Microphotograph showing a severely infected middle ear cavity. The inner ear is well protected by footplate (F). Horizontal section. Hematoxylin-eosin staining. 27 X.

- X - External auditory meatus
- S - Saccule
- Z - Vestibular nerve
- C - Cochlea



Figure 4

Microphotograph demonstrating another view of very severe middle ear infection. The inner ear is protected by round window membrane (large arrow). Small arrow indicates cochlear aqueduct. Note some precipitate in scala vestibuli and tympani. The external auditory meatus (X) is clean. Horizontal section. Hematoxylin-eosin staining. 27 X .



Figure 5

Microphotograph showing infection-free ear apparatus. Notice the large stapedia artery (small arrow) and flocculus cerebelli (large arrow). Horizontal section. Hematoxylin-eosin staining. 19 X.

- X - External auditory meatus
- T - Tympanic cavity
- M - Malleus
- K - Incus
- F - Footplate of stapes
- C - Cochlea
- S - Saccule



Figure 6

High magnification view of the internal auditory meatus. An arrow indicates the migrated nuclei cells in the vestibular nerve. Horizontal section. Hematoxylin-eosin staining. 90 X.

- S - Saccule
- B - Organ of Corti in the basal turn
- N - Cochlear nerve
- G - Vestibular ganglion (Scarpa's ganglion)

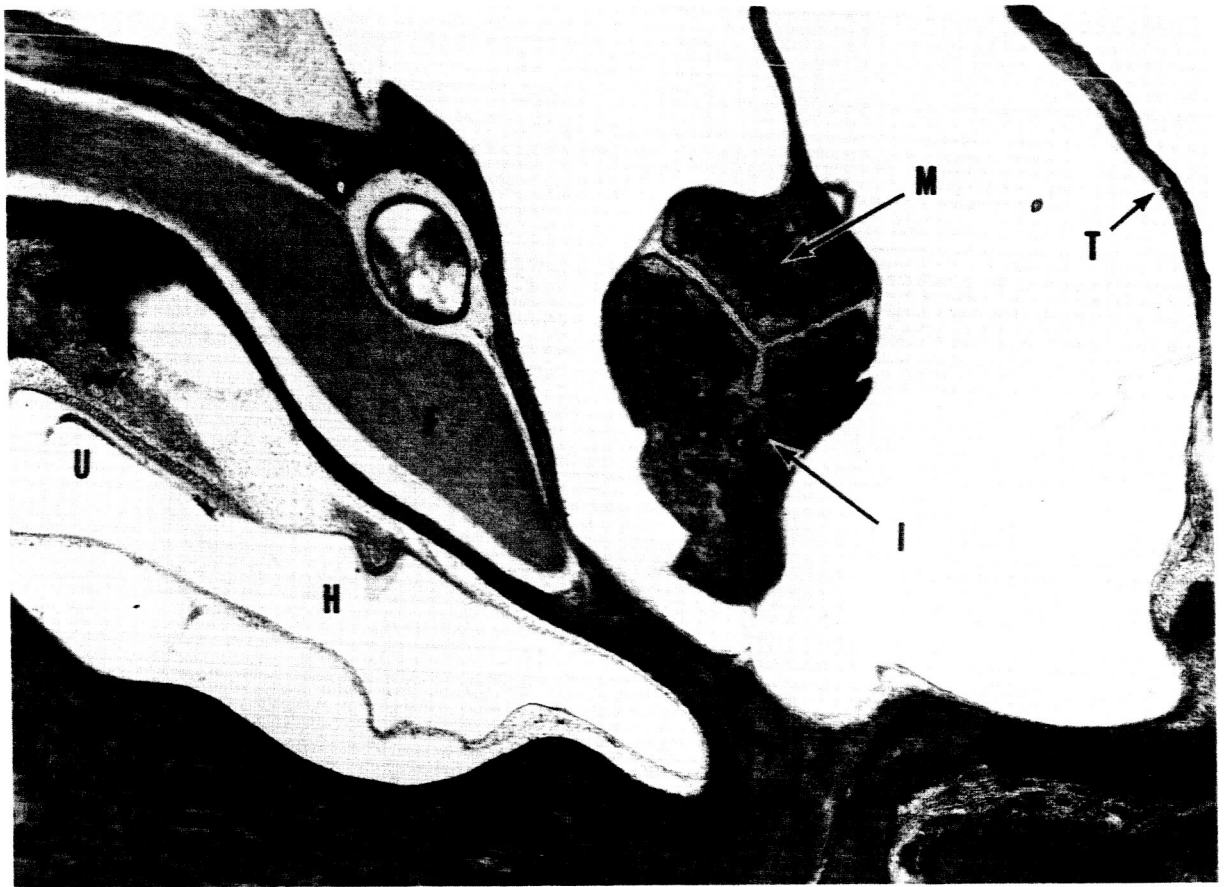


Figure 7

Microphotograph showing infection-free middle and inner ear. Horizontal section. Hematoxylin-eosin staining. 55 X.

T - Tympanic membrane

M - Malleus

I - Incus

F - Facial nerve

H - Horizontal semicircular canal crista

U - Macula utriculi

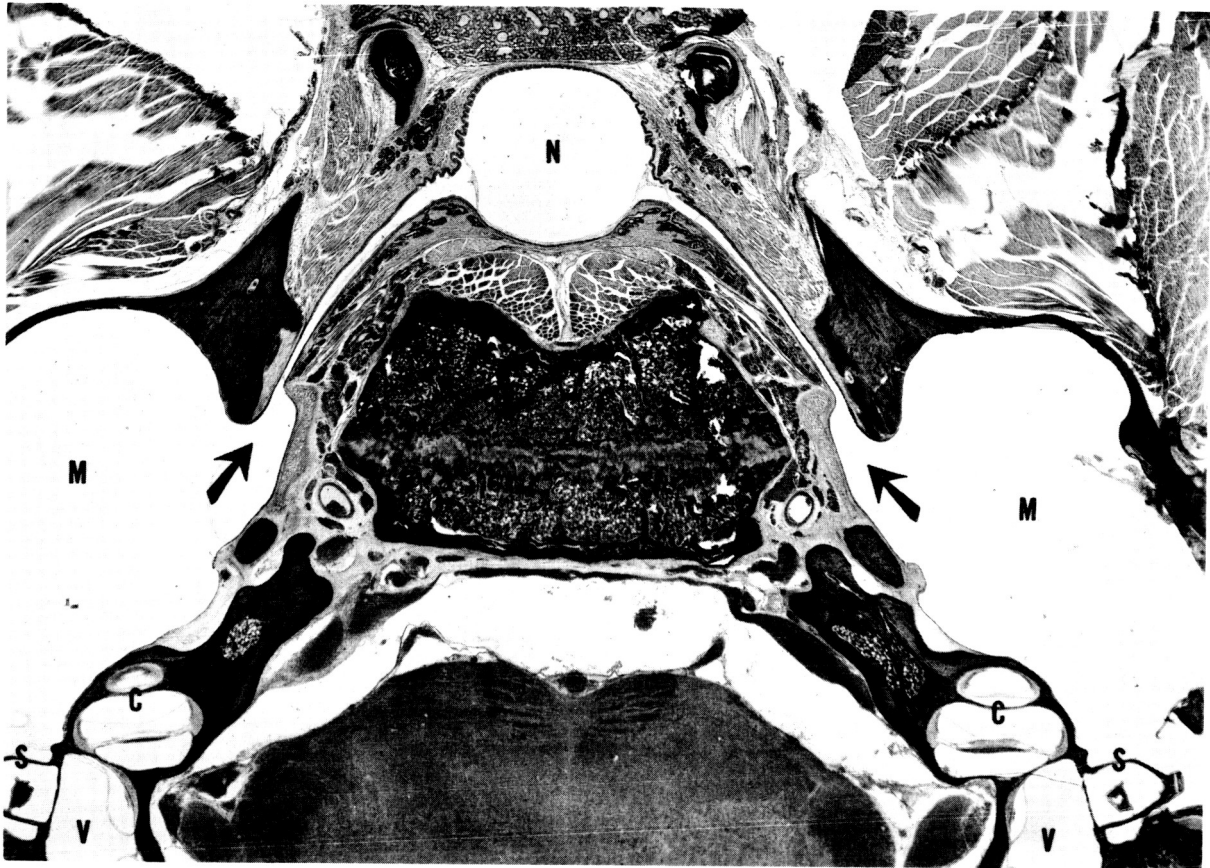


Figure 8

Microphotograph demonstrating the wide opened Eustachian tubes in an infection-free ear. The arrows indicate tympanic orifice of the tube. Horizontal section.

Hematoxylin-eosin staining. 16 X.

- N - Nasopharynx
- M - Middle ear cavity
- C - Cochlea
- V - Vestibule
- S - Stapes

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<p>The ears of sixteen albino rats originating from three different suppliers were histologically examined. The specimens were prepared following the standard temporal bone preparation procedure. Spontaneous middle ear infection was found in 25% of the ears.</p> <p>When albino rats are used for otological experiments, it is necessary to have a large number of experimental animals as there is no precise method for detection of spontaneous ear infection of the rat in the vital status. Post-mortem histological investigation is the only reliable procedure available by which otological infections can be detected.</p>		

14. KEY WORDS	LINK A		LINK B		LINK C	
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